INFLUENCE OF TEMPERATURE AND SPORE STAGE ON PRODUCTION OF TELIOSPORES BY SINGLE AECIOSPORE LINES OF *CRONARTIUM RIBICOLA*

Geral I. McDonald and Duane S. Andrews

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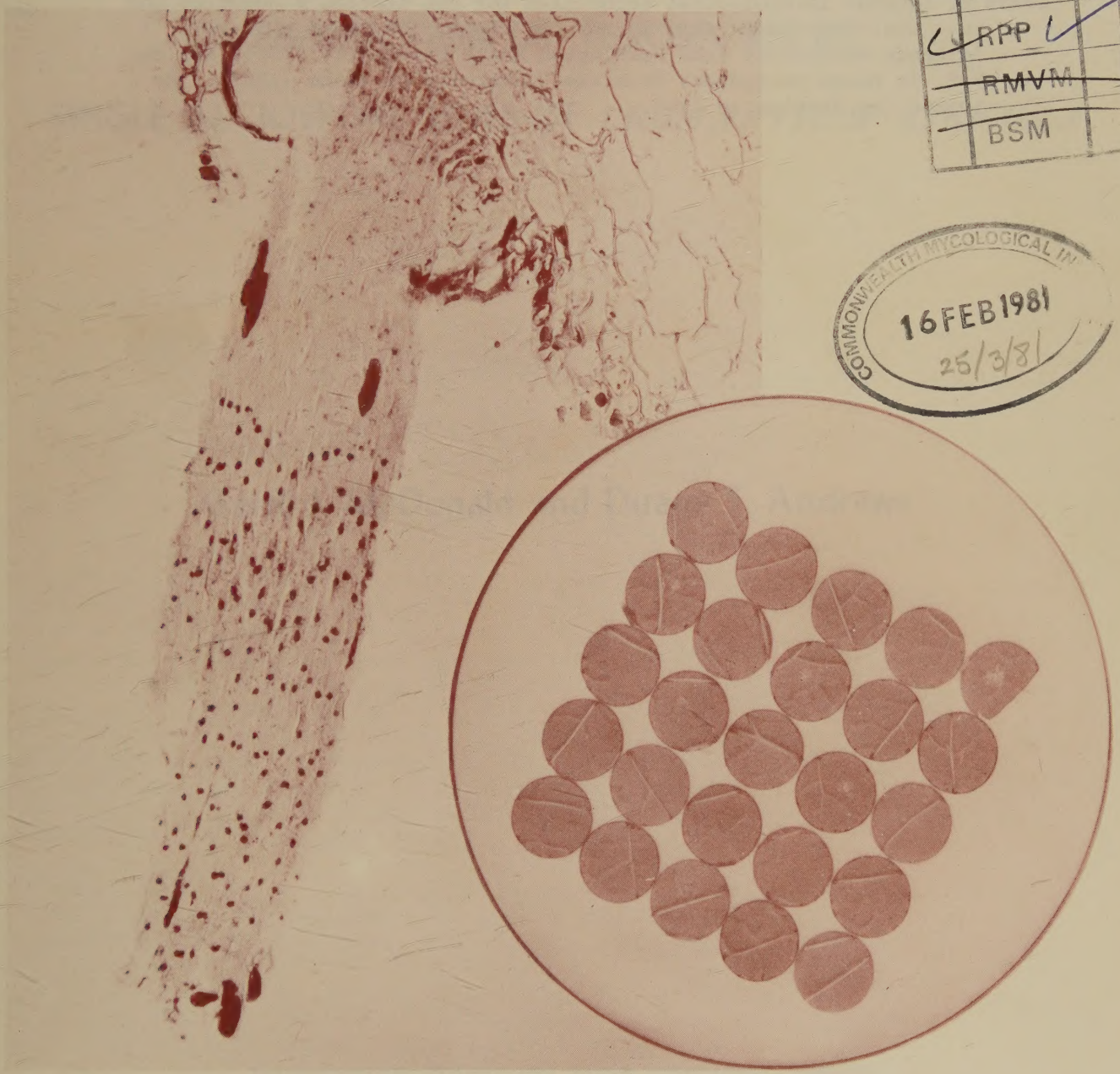

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RESEARCH SUMMARY

A major problem facing those who wish to inoculate white pines with *Cronartium ribicola* is the reliable production of teliospores. Also, a better understanding of the nature of the various spore stages will add to our ability to develop workable integrated rust management plans.

The results reported in this paper will increase the reliability of teliospore production in both whole plant and detached-leaf-culture of *Cronartium ribicola* on Ribes plants, and add to our understanding of the interaction between genes and the environment in the functioning of epidemiological fitness traits.

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INTRODUCTION

Over the last few years, attempts have been made to establish single aeciospores lines of *Cronartium ribicola* to facilitate the investigation of the genetics of this important conifer rust. A principal objective was to complete the life cycle in isolation so that known homozygous aeciospores could be produced. A modified version (McDonald 1978) of Clinton and McCormick's (1924) Petri dish method has worked well for the ribes portion of the life cycle. But lack of consistent and profuse production of teliospores has been an unexpected problem. Past greenhouse studies show that a constant 61°F (16°C) from inoculation to spore production led to abundant telia (Riker and others 1947 and Van Arsdel and others 1956). Also, telia would not form if the day and night temperature was above 68°F (20°C) (Van Arsdel and others 1956).

Our first problem arose when a group of single aeciospore lines isolated at a constant 70°F (21°C) were transferred to a constant 55°F (13°C). One culture out of 69 produced telia within 60 days (author's unpublished data). On the other hand, other single aeciospore cultures held at 70°F±2 (21°C±1) produced teliospores that would germinate as they formed (McDonald and Andrews, in preparation). So, a reliable supply of ungerminated teliospores to use for inoculation back to *Pinus monticola* proved difficult to obtain. A series of experiments were initiated to determine the best procedure for producing teliospores on the detached leaf cultures.

Two pieces of information were used to formulate a hypothesis. First, we observed that when inoculations were made with urediospores instead of aeciospores, we could obtain teliospores in as little as 14 days at 70°F (21°C) and 16 hr days (author's unpublished data). Whereas, aeciospore inoculations would nearly always require 28 to 30 days for earliest appearance of telia, regardless of temperature and day length. Second, Spaulding (1922) reported that Pennington and Snell followed generations of urediospore production in the field. Teliospores were not produced with the first generation but were found with all later generations. We hypothesized that aeciospore and urediospore infections differed in their ability to produce teliospores and that a temperature of less than 68°F (20°C) was needed to stimulate teliospore production by infection resulting from urediospores. The objective of this paper is to report the results of initial experiments designed to test the above hypothesis by growing both urediospore and aeciospore stages of identical genetic background on one clone of ribes in one growth chamber (urediospore lines compared to the aeciospore line from which they decended). Thus, as near as is currently possible, only spore stage varied.

MATERIALS AND METHODS

We used two sets of materials, one isolated in 1976 and the other in 1979. Both consisted of single aeciospore lines isolated according to McDonald and Andrews (in preparation). Upon production of urediospores, subcultures were established. All cultures of both spore types were grown on *Ribes hudsonianum* var. *petiolare* clone INT-1. The 1976 material has already been described (McDonald and Andrews, in preparation) and was included in this paper to provide some comparisons with other sources of aeciospores. The 1979 material was produced and the experiment conducted as outlined in fig. 1. Two cankered *P. monticola* seedlings growing in the greenhouse were producing aeciospores. Spores from one blister on each tree were used as follows: About half of the 1,058 attempted isolations were made from each blister, and isolated spores were randomly placed in a cool 55°F±2 (13°C±1), and a warm 70°F±2 (21°C±1), chamber as they were isolated (16 hr day 8 hr night).

The aeciospore cultures were inspected every 7 days for 65 days, and presence of urediospores and teliospores was recorded. Six subcultures were attempted from all the single aeciospore cultures that had produced sufficient urediospores by 21 days. These cultures are a random selection of possible cultures. One-half of these subcultures were placed in the warm chamber and one-half in the cool chamber from each of the warm and cool cultures from which they were isolated (fig. 1). These cultures were then inspected every 7 days for 49 days and presence of urediospores and teliospores was recorded.

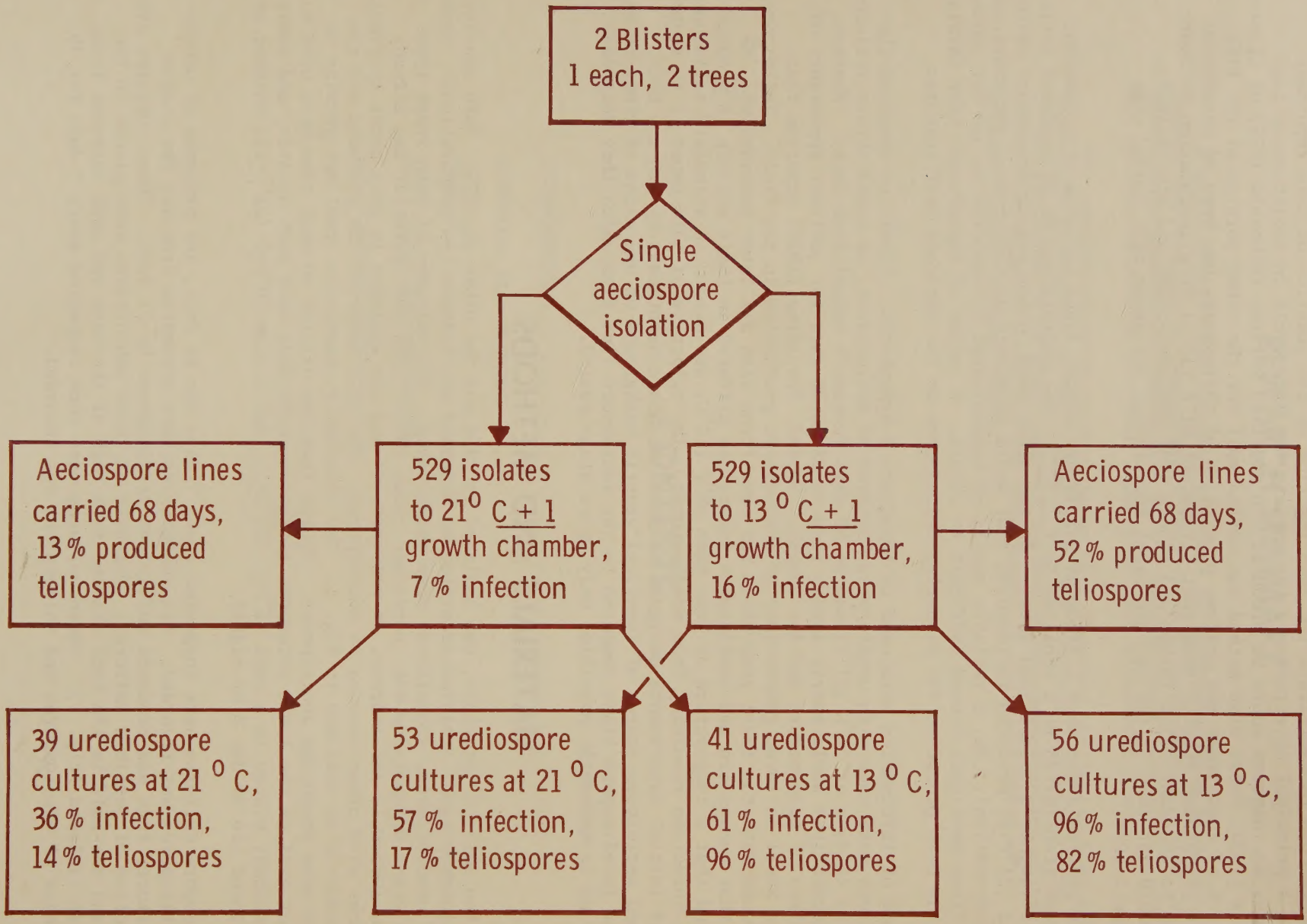

Figure 1.--Layout and sketch results of *Cronartium ribicola* teliospore production experiment. All cultures propogated on greenhouse grown leaves of *Ribes hudsonianum* var. *petiolare* detached at 21 days of age and placed in 9 cm Petri dishes placed in one of two growth chambers (16 hr day 8 hr night).

We used chi-square analysis (Snedecor 1956) to test for independence of selected comparisons of spore type, spore source, and temperature for inoculation success and presence of teliospores. In addition, the pattern of subculture age at teliospore appearance was recorded for all cool chamber aeciospore lines that produced three subcultures.

RESULTS

As expected, the single aeciospore isolates placed in the cool chamber provided a higher success rate than those placed in the warm chamber (table 1). But the warm chamber isolates developed slightly faster. All the infections that appeared by 65 days were evident in 14 days in the warm chamber; but cool chamber infections were not evident until the 21 day inspection.

There was a significant difference due to temperature in teliospore production by 49 days by aeciospore cultures (table 2). The cool chamber isolates showed a significant increase in percent producing telial columns.

Table 1.--*Percent of single aeciospores of* Cronartium ribicola *that produced infections on leaf disks of* Ribes hudsonianum *var.* petiolare *within 21 days when grown in a cool and a warm chamber, both under 16 hr days*

Chamber temperature	Infections Yes	No	Total	Percent
55°F±2 (13°C±1)	86	443	529	16
70°F±2 (21°C±1)	35	494	529	7
Total	121	937	1058	11

$\chi^2 = 24.28$ P of larger $\chi^2 = 0.0005$
d.f. = 1

Table 2.--*Percent of single aeciospore cultures of* Cronartium ribicola *that produced teliospores by 49 days after inoculation when grown on* Ribes hudsonianum *var.* petiolare *INT Clone-1 at cool and warm temperatures under 16 hr days*

Chamber temperature	Teliospores Yes	No	Total	Percent
55°F±2 (13°C-1)	43	39	82	52
70°F±2 (21°C±1)	2	13	15	13
Total	45	54	97	46

$\chi^2 = 7.75$ P larger $\chi^2 = 0.01$
d.f. = 1

Regarding urediospore inoculation success, the cool chamber was best regardless of temperature of parent culture's chamber (tables 3 and 4).

Table 3.--*Percent of* Cronartium ribicola *urediospore inoculations that produced infection in 21 days. Urediospores obtained from a population of single aeciospore cultures grown on* Ribes hudsonianum *var.* petiolare *INT Clone-1 leaf disks in a 70°F±2 (21°C±1) chamber and inoculated back to INT Clone-1 in 55°F±2 (13°C±1) and 70°F±2 (21°C±1) chambers with all chambers at 16 hr days*

Treatment	Infections Yes	No	Total	Percent
Aeciospore culture to Urediospore subculture				
Warm to cool	25	16	41	61
Warm to warm	14	25	39	36
Total	39	41	80	49

$\chi^2 = 5.01$ P of a larger $\chi^2 = 0.025$
d.f. = 1

Table 4.--*Percent of* Cronartium ribicola *urediospore inoculations that produced infections in 21 days. Urediospores obtained from a population of single aeciospore cultures grown on* Ribes hudsonianum *var.* petiolare *INT Clone-1 leaf disks in a 55°F±2 (13°C±1) growth chamber and inoculated back to INT Clone-1 in 55°F±2 (13°C±1) and 70°F±2 (21°C±1) chambers with all chambers at 16 hr days*

Treatment	Infections Yes	No	Total	Percent
Aeciospore culture to Urediospore subculture				
Cool to cool	54	2	56	96
Cool to warm	30	23	53	57
Total	84	25	109	77

$\chi^2 = 24.41$ P of larger $\chi^2 = 0.0005$
d.f. = 1

In the case of teliospore production by urediospore cultures, the cool chamber produced a higher percentage of telial supporting cultures regardless of the temperature at which the urediospores were produced (tables 5 and 6). A feature of importance to us was culture age at teliospore appearance. The cool chamber produced teliospores more quickly than the warm chamber (tables 7 and 8), but a statistical test could not be performed because of the low level of teliospore production in the warm chamber. The temperature of the chamber that produced the urediospores had no apparent influence (table 9).

Table 5.--*Percent of* Cronartium ribicola *urediospore cultures derived from population of single aeciospore cultures grown in warm chamber on* Ribes hudsonianum *var.* petiolare *INT Clone-1 that produced teliospores by 49 days after inoculation when grown on Clone-1 at 55°F±2 (13°C±1) and 70°F±2 (21°C±1) and 16 hr days*

Treatment	Teliospores		Total	Percent
	Yes	No		
Aeciospore culture to Urediospore subculture				
Warm to cool	22	1	23	96
Warm to warm	2	12	14	14
Total	24	13	37	65

$\chi^2 = 25.27$ P of larger $\chi^2 = 0.0005$
d.f. = 1

Table 6.--*Percent of* Cronartium ribicola *urediospore cultures derived from population of single aeciospore cultures grown in cool chamber on* Ribes hudsonianum *var.* petiolare *Clone INT-1 that produced teliospores by 49 days after inoculation when grown on Clone-1 at 55°F±2 (13°C±1) and 70°F±2 (21°C±1) and 16 hr days*

Treatment	Teliospores		Total	Percent
	Yes	No		
Aeciospore culture to Urediospore subculture				
Cool to cool	41	9	50	82
Cool to warm	5	24	29	17
Total	46	33	79	58

$\chi^2 = 31.67$ Probability of larger $\chi^2 = 0.0005$
d.f. = 1

Table 7.--*Number of* Cronartium ribicola *urediospore cultures derived from a population of single aeciospore cultures grown in cool chamber on* Ribes hudsonanium *var.* petiolare *INT Clone-1 that produced teliospores at various times after inoculation when grown at two different temperatures on INT Clone-1*

Treatment	Days after inoculation 21	28	35	49	Total
Aeciospore culture / Urediospore to subculture					
Cool to cool	19	18	0	4	41
Cool to warm	0	0	1	4	5
Total	19	18	1	8	46

Table 8.--*Number of* Cronartium ribicola *urediospore cultures derived from a population of single aeciospore cultures grown in warm chamber on* Ribes hudsonianum *var.* petiolare *INT Clone-1 that produced teliospores at various times after inoculation when grown at two different temperatures on INT Clone-1*

Treatment	Days after inoculation 21	28	35	49	Total
Aeciospore culture / Urediospore to subculture					
Warm to cool	7	12	1	2	22
Warm to warm	1	0	0	1	2
Total	8	12	1	3	24

Table 9.--*Number of* Cronartium ribicola *urediospore cultures derived from two populations of single aeciospore cultures, one grown in a* $70^{\circ}F \pm 2$ ($21^{\circ}C \pm 1$) *chamber, the other grown in a* $55^{\circ}F \pm 2$ ($13^{\circ}C \pm 1$) *chamber and both grown on* Ribes hudsonianum *var.* petiolare, *that produced teliospores at various times after inoculation when grown at* $55^{\circ}F \pm 2$ ($13^{\circ}C \pm 1$) *on INT Clone-1*

Treatment	Days after inoculation 21	28	35	49	Total
Aeciospore culture / Urediospore to subculture					
Warm to cool	7	12	1	2	22
Cool to cool	19	18	0	4	41
Total	26	30	1	6	63

$\chi^2 = 2.95$ P of larger $\chi^2 = 0.40$
d.f. = 3

Both initial aeciospore cultures and their derived urediospore cultures yielded a high proportion of cultures producing teliospores when grown in a cool temperature, but a most important difference showed up when culture ages were compared (table 10). Few aeciospore cultures produced teliospores in less than 28 days, whereas nearly all the genetically related urediospore subcultures had produced their teliospores by 28 days.

We conducted our first test of temperature effects on urediospore subcultures (1/single aeciospore culture) for another population of aeciospore cultures isolated in 1976. When we compared the 1976 and 1979 subcultures, a highly significant difference existed between the two populations (table 11), but both populations tended toward the early production pattern.

The 1976 and 1979 aeciospore cultures were also compared because we had no previous data on aeciospore cultures grown in both warm and cool chambers. The result was consistent (table 12) in that the aeciospore cultures in the cool chamber produced telia before those in the warm chamber. Also, both were consistent in not producing teliospores at an early age.

Finally, most urediospore subcultures within a single aeciospore line showed little variation in age at teliospore appearance, and 2 (C-9 and C-70) of the 18 lines that had complete sets of 3 subcultures failed to produce any teliospores by 49 days (table 13).

Table 10.--*Number of single aeciospore cultures and urediospore subcultures of* Cronartium ribicola *that produced teliospores at various times after inoculation when grown on* Ribes hudsonianum *var.* petiolare *clone INT-1 at* $55^{o}F \pm 2$ *(*$13^{o}C \pm 1$*)*

Inoculation spore type	Days after inoculation 21	28	35	49	Total
Aeciospores	0	2	11	45	58
Urediospores	19	18	0	4	41
Total	19	20	11	49	99

$\chi^2 = 76.52$ P of larger $\chi^2 = 0.0005$
d.f. = 3

Table 11.--*Number of* Cronartium ribicola *urediospore subcultures from two populations of single aeciospore cultures that produced teliospores at various times after inoculation when growing at* $55^{o}F \pm 2$ *(*$13^{o}C \pm 1$*) (16 hr days) on* Ribes hudsonianum *var.* petiolare INT Clone-1

Subculture source	Days after inoculation 21	28	35	Total
1976 Single aeciospore culture	39	83	49	171
1979 Single aeciospore culture	19	18	0	37
Total	58	101	49	208

$\chi^2 = 19.49$ P of larger $\chi^2 = 0.0005$
d.f. = 2

Table 12.--*Number of single aeciospore cultures of* Cronartium ribicola *growing on INT Clone-1 of* Ribes hudsonianum *var.* petiolare *that produced telial columns at various times after inoculation contrasting 1976 isolates grown at 70°F±2 (21°C±1) with 1979 isolates grown at 55°F±2 (13°C±1)*

Isolate population	Days after inoculation 21	28	35	65	Total
1976 warm aeciospores	0	3	20	202	225
1979 cool aeciospores	0	2	11	45	58
Total	0	5	31	247	283

$\chi^2 = 11.48$ P of larger $\chi^2 = 0.01$
d.f. = 3

Table 13.--*Patterns of variation within 18 single aeciospore lines and among subcultures within lines in days to appearance of teliospores. Cultures were growing on detached leaves of* Ribes hudsonianum *var.* petiolare *at 55°F±2 (13°C±1) (16 hr days) for 49 days*

	Single Aeciospore Lines (C = cool; W = warm)																
Urediospore Subculture	C-1	C-26	W-2	W-3	C-42	C-44	C-35	C-18	W-4	C-50	W-34	C-31	C-63	C-22	C-71	W-7	C-9[1/] C-70[2/]
1	21	21	21	21	21	21	21	21	21	21	28	28	28	28	28	28	0 0
2	21	21	21	21	21	21	21	21	28	49	20	20	20	20	20	20	0 0
3	21	21	21	28	28	28	28	49	28	0	28	28	49	49	0	0	0 0

[1/] This parent aeciospore culture produced T in 56 days.
[2/] This parent aeciospore culture produced T in 48 days.

DISCUSSION AND CONCLUSION

Cultures of *C. ribicola* obtained from aeciospores or urediospores differ in patterns of teliospores production. However, one cannot say if most aeciospore-derived infections are unable to produce the teliospore stage or if it just takes longer. The answer could come from experiments designed to allow the aeciospore derived cultures to grow but to prevent all possibility of urediospores reinfecting the leaves on which they were produced.

Another conclusion is that temperatures near 55°F (13°C) stimulate both infection success and teliospores production for both aeciospore- and urediospore-derived detached leaf cultures of *C. ribicola*.

The scenario suggested here may explain the pattern of teliospore production obtained by Clinton and McCormick (1924). They made several hundred aeciospore and urediospore inoculations over 3 years on detached leaves of many different ribes species. Their cultures were grown in a greenhouse, so they were not able to exercise much control over temperature. Over years they attempted 131 inoculations with aeciospores (not single spores) during April, May, and June and obtained 83 infections (63 percent success). Of the 83 infections, 2 (2 percent) produced teliospores within 3 weeks (their cultures apparently did not live much beyond 3 weeks although they did not explicitly state any average age). In addition, they obtained about 47 infections from urediospores. Of these, 34 were inoculated between September 13 and 17, 1918, of which 22 (65 percent) produced teliospores. Of 49 infections obtained from urediospore inoculation during April, May, June, July, and August, only 14 percent produced teliospores. If we assume that their cultures lasted an average of 21 days and that only the September 13 to 17, 1918 period was cool enough, our results could be interpreted as being in total agreement with theirs.

New attempts at isolation of single aeciospores should be conducted at a cool temperature, 55°F (13°C). Time required to complete the life cycle in isolation can be reduced by reinoculating with urediospores as soon as they are produced. Also, early teliospore production by certain single aeciospore-derived infections may indicate the existence of yet another *C. ribicola* marker gene as well as having possible important significance to the epidemiological relationships of *C. ribicola*. Other rust species may have responded in a like manner (Savile 1953) in their adaptation to short seasons. Genetic control of a trait such as teliospore production by infections arising from different spore stages as well as variation among individual spores could indicate the operation of a fundamental adaptive device that might have its ultimate expression in the formation of microcyclic races.

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Genetically identical aeciospore and urediospore populations grown on a single ribes clone varied in time required to produce teliospores. Infection developing from urediospores usually produced teliospores in less than 28 days when grown at 55°F (13°C); whereas, infections developing from aeciospore generally required more than 35 days at the same temperature.

KEYWORDS: *Cronartium ribicola*, epidemiological fitness, inherited traits, detached leaf culture of rust

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